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Original Article

The Vitamin D Receptor (VDR) Gene Polymorphisms Fok1 In Obese Pakistanis – a Preliminary Report

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ABSTRACT

Objective: The role of vitamin D has soared to a pinnacle in recent years with functions affecting 229 human genes linked to cardiovascular, autoimmune, humoral, pulmonary and neurological diseases. Relationship between obesity and vitamin D has not as yet been fully established. The objective of the study was to determine the possible relationship between Vitamin D receptor (VDR) gene polymorphisms and the risk of obesity. **Methods:** Total 100 subjects (50 obese and 50 normal) were selected. Oral rinse samples were collected (between 18-45 years of age) after an informed consent. DNA was extracted and PCR was performed using VDR-Fok1 primers. A 256bp amplified products was visualized by Gel Doc Hero Lab software (Germany). **Results:** The results of VDR-Fok1 gene polymorphism showed that out of 50 obese subjects 40% were normal (FF=20), 54% were Heterozygous (Ff = 27) and 6% were mutated (ff= 3). Out of 50 non-obese subjects 84% were normal (FF= 42), 16% were Heterozygous (Ff= 8) and none were mutated (ff= 0%). The statistical analysis results between the ff genotype and obesity were not significant. The ff genotype of VDR may be associated with obesity. More studies with larger sample size are required to find a stronger correlation.

1. INTRODUCTION

The increasing prevalence of obesity worldwide especially in the pediatric age group is a cause of a major global concern. Studies from physical activity to various metabolic pathways in the body are being conducted all over the world to find links that would help to prevent obesity in the population in general (Crawford D, 2002). Since the role of vitamin D has expanded to new hypothetical levels stretching beyond calcium homeostasis to cardiovascular, autoimmune, tumoral, pulmonary and neurological diseases, showing links with 229 human genes. It is imperative to analyze the link between vitamin D and obesity. In southeast Asia it has been found out that people at all levels suffer from vitamin D deficiency in spite of plentiful sunlight.

Therefore, it is essential to find out the clinical and physiological significance of genotypes of vitamin D and their relationship with molecular and functional aspects of polymorphisms in our population (Baig S et al 2010). Genetic variation in the VDR has been found correlated with Body mass measurements (fat mass, lean mass, body weight and body mass index) and muscle strength (quadriceps, hamstring and grip strength) in a Swedish study (Grundberg E et al 2004). Obesity is an excess proportion of total body fat. Several More defined phenotypes have been proposed for studying obesity, such as fat mass, lean mass, and percentage fat mass. There are several risk factors for obesity that may differ across patients, namely age, gender, density of adipose tissue, menopause, genetic risk factors or hormones like Leptin (Farooqi IS et al 2002). A functional genetic

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polymorphism in *VDR* could be involved in other tissues that respond to vitamin D, such as muscle cells and adipocytes. Indeed, it has been demonstrated that vitamin D stimulates the differentiation of preadipocytes to adipocytes in the OB17 cell line (Dace A et al 1997). *VDR* genotype has also been suggested as a determinant of body composition by some studies (Grundberg E et al 2004). This study was designed to determine the possible relationship between Vitamin D receptor (*VDR*) gene polymorphisms and the risk of obesity.

2. MATERIALS AND METHODS

This case control study was conducted in Research Laboratories Ziauddin University, Karachi.

2.1. Samples collection

Oral rinse samples were collected after taken informed consent prior to sampling from 50 (25males and 25 females) obese individuals whose BMI was greater than 30, and 50 non obese (25males and 25 females) age matched with BMI 25 or less. Individuals included were

healthy adults between 20-45 years of age. The procedure devised to get more of DNA was a use of toothpick with a small bristle on the other end used for dental floss. The subjects, after collection of oral rinse, were asked to swipe the bristle on the oral mucosa of cheeks to gather a good number of mucosal cells.

2.2. DNA extraction

The genomic DNA was isolated from oral rinse according to Lucky MH et al 2013. The DNA was quantified by using Qubit® dsDNA BR Assay (Qubit® 2.0 invitrogen life technologies USA) and the quality was checked by running an aliquot on 0.6% agarose gel stained with ethidium bromide

2.3. Polymerase chain reaction

Reaction mixtures of 50 µl were used in PCR for the *VDR* gene (*FokI*) polymorphism. The DNA samples were amplified in BioFlux Thermal Cycler (BioFlux, Korea). The primers used were as reported earlier for *FokI* (Harris SS et al 1997).

Table1.

Sequences of *FokI* primers

Gene	Primer sequence	PCR product			
		265bp	FF	Ff	ff
Chromosome 12	Forward 5'-AGCTGGCCCTGGCACTGACTCTGCTCT-3'	265bp	265bp	265,196,69bp	196, 69 bp
Exon2 (T/C)	Reverse 5'-ATGGAAACACCTTGCTTCTTCTCCCTC-3'				
<i>FokI</i>					
rs2228570					

2.4. PCR Cycling Conditions

2.4.1. *VDR FokI*

DNA samples were amplified with cycling parameters as follows: Initial denaturation at 94°C for 5 minutes followed by 35 cycle of 94°C for 45 seconds, 58°C for 45 seconds, followed by 74°C for 45 seconds, and a final extension at 74°C for 3 minutes. The PCR reaction was carried out in 50 µl volume, containing 25 µl of GoTaq® Green master mix (GoTaq® DNA Polymerase is supplied in 2X Green GoTaq® Reaction Buffer pH 8.5, 400 µM dATP, 400 µM dGTP, 400 µM dCTP, 400 µM dTTP and 3 mM MgCl₂, Promega, USA) 4 µl of 1 µM of each primer (Genelink, USA), 5 µl (100-200 ng) of DNA template and

12 µl of PCR graded water (Promega, USA). A 265bp Bands were visualized on Hero Lab (Germany) Gel Doc Imaging system.

2.4.2. *FokI* polymorphism

The 0.2ul of PCR product was digested with 1 µL of Fast Digest *FokI* restriction enzyme (Fast Digest *FokI* Fermentas, USA) and the 1X FastDigest buffer and incubated at 37°C for 5 minutes. The ff genotype (homozygote of infrequent allele) generated two fragments of 196 and 69 bp. The heterozygote displayed three fragments of 265, 196 and 69 bp, designated as Ff.

2.5. Statistical Analysis

Statistical analysis was done with SPSS version 20.0 (IBM, SPSS Statistics 20.0). Allele frequency was calculated as the number of occurrences of the test allele in the population divided by the total number of alleles. The association of obese and non-obese group with different genotypes of Fok1 was assessed by Chi square test.

3. RESULTS

Out of 100 samples (50 obese and 50 non-obese) the PCR-RFLP results showed (Table. 2) that FF genotype (homozygote of common allele) with one band of 265bp. The ff genotype (homozygote of infrequent allele) generated two fragments of 196bp and 69bp. The heterozygote displayed three fragments of 265, 196 and 69bp, designated as Ff. Chi square test was applied The p value is significant with association of FF and Ff with obese and non-obese subject but shows no significant with ff genotype. The PCR-RFLP results showed (Table. 2) the homozygote of common allele the FF genotype as 40% (20) with one band of 265bp. The homozygote of infrequent allele, the ff genotype was 6% (3) which generated two fragments of 196bp and 69bp. The Ff heterozygote genotype was 54% (27) which displayed three fragments of 265, 196 and 69bp.

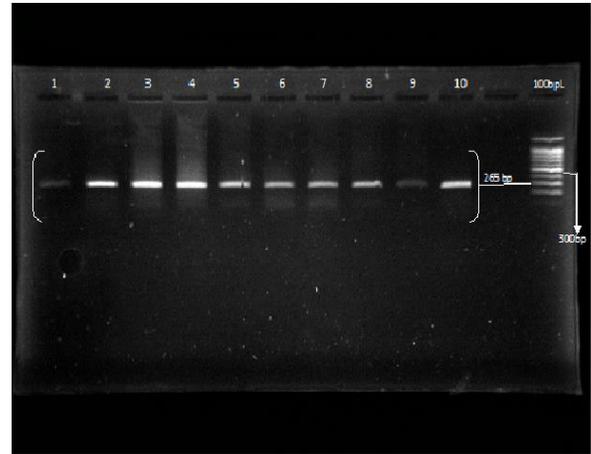


Figure 1: Lane 1-10 showing VDR fok-1 gene band of 265bp.

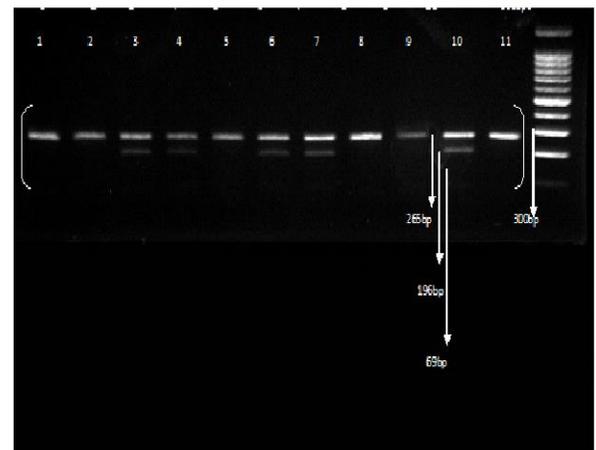


Figure 2. Lane 1, 2, 5, 8, 9 and 11 shows FF genotype whereas lane 3, 4, 6, 7 and 10 shows Ff genotype of VDR fok-1 gene polymorphism.

Table2.

Frequency of Fok1 polymorphism and allelic frequency (C\T) in normal and obese subjects

Classification	BMI	Frequency of Fok1 polymorphism			Allele frequency (C/T) of Fok 1 polymorphism	
		FF	Ff	ff	p (C allele)	q (T allele)
Obese (n= 50)	≥ 30	20(40%)	27(54%)	3(6%)	(0.67)	(0.33)
Non obese (n = 50)	≤ 25	42(84%)	8(16%)	0(0%)	(0.92)	(0.08)
		(<i>P= 0.000</i>)	(<i>P= 0.000</i>)	(<i>P= 0.079</i>)		

The allele distribution showed higher frequency of the wild F allele in non obese group and the minor ff homozygous was only found in obese group. Chi-square test was applied on the obese and non-obese subjects and significant difference in the frequency distribution of both genotypes and alleles were observed in almost all subjects, the wild homozygous FF genotype was present in 40% obese versus 84% non obese while the minor ff homozygous genotypes was found in 6% obese versus 0% of non obese and the heterozygous genotypes was found in 54% obese versus 16% non obese. The p-value was significant for FF and Ff but not significant for ff.

4. DISCUSSION

The importance of the *vitamin D* in bone biology is highly accredited, but its importance to obesity has been rarely investigated. The results of this preliminary study show that frequency of VDR Fok1 polymorphism, genotype ff is 6% in obese subjects in our population. It was also observed that a larger proportion of obese subjects had heterozygous Ff gene compared to their non-obese counterparts. This was also significantly different from the non-obese subjects. Low vitamin D levels have been found associated with Total abdominal adipose tissue at the L2-L3 intervertebral level (Bhatt SP et al 2014). Many such studies and reviews have been previously published on low vitamin D but very few of them have focused on the effects of vitamin D polymorphism in causing obesity in individuals. Obesity is an excess proportion of total body fat and body weight is the most common index of obesity. It is well known that the larger the body mass the greater is the mechanical load on the bones. Body mass index (BMI) is widely used as an index of the degree of obesity, primarily because it is easy to measure, but it cannot be used to distinguish body fat from lean mass. In this study BMI was 30. Polymorphism in VDR TaqI 'T' allele has been found to contribute to an elevated BMI of 3 kg/m² per risk allele (OR: 2.07; 1.123–3.816; *P* = 0.019) suggesting a role for VDR as risk factor for obesity (Vasilopoulos Y et al 2013). Obesity with its mechanical loading effects of total body weight on bone mass may be

a cause of osteoporosis. Obesity and osteoporosis could be closely related both having multifactorial etiologies including genetic and environmental components, with potential interactions between them (Zhao LJ et al 2007). Since osteoporosis is linked with both VDR polymorphism and obesity a Chinese study was conducted to find a link between the two, VDR polymorphism and obesity (Jie-mei Gu et al 2009). Gu et al found association of VDR polymorphisms with total fat mass in young Chinese men which constituted whole body fat mass, lean mass, and FPM as indices of the degree of obesity, using DXA. The (VDR) receptor gene is one of the most extensively studied genes in relation to Bone Marrow Density (Uitterlinden A et al 2010). Since both bone mass and obesity phenotypes are known to be under strong genetic regulation (Zhang ZL et al 2008) the whole-genome linkage scan has identified several genomic regions shared by obesity and osteoporosis (Tang ZH et al 2007). Generally the role of Vitamin D is described as a transcription factor that influences central mechanisms of tumorigenesis: growth, cell differentiation, and apoptosis (Vuolo L et al 2012). Fok1 VDR polymorphism has been found in number of studies to be more frequently associated with tumorigenesis; though their role in the cancer is not yet fully understood. The results of a meta-analysis show that Fok1, may be one of the susceptibility biomarker for skin cancer in Caucasians (Zhao XZ et al 2014). Another meta-analysis of studies showed that the Fok1 polymorphism

of the VDR gene was associated with an increased risk of breast cancer (ff vs. Ff+FF, OR: 1.09, 95%CI: 1.02 to 1.16, p=0.007) (Zhang K et al 2014). When correlation between vitamin D status, gene expression in immune cells and genetic variants were investigated in healthy controls (HC) it was found that both D3 deficiency and low D3 levels appear to interact with its system gene transcription illustrating the relevance for targeted vitamin D therapy (Morán-Auth Y et al 2013). Since Vitamin D deficiency may be associated with the cardio-metabolic risk profile in obese people, genetic variation at the Fok1 polymorphism of the VDR gene, in combination with vitamin D levels, was investigated to be found associated with plasma renin activity in hypertension. This makes the vitamin D-VDR complex as a potential regulator of renin activity in humans and further suggest that vascular RAS activity may progressively increase when 25(OH)D deficiency occurs in obesity (Vaidya A et al 2010). Prospective studies possibly will be able to establish the role and amount of Vitamin D supplementation in obese individuals at increased cardiovascular risk. VDR polymorphism studies on Vitamin D show that deficiency of vitamin D3 is beyond exposure to sun. Researchers (Theuri G et al 2013) feel that there is likely a global epidemic of Vitamin D deficiency and fortification of foods and beverages with Vitamin D3 will soon be required for populations found compromised genetically or nutritionally.

CONCLUSION

The ff genotype of VDR may be associated with obesity. More studies with larger sample size are required to find a stronger correlation. Confirmations of these results are required in our and other populations with more functional markers of the VDR.

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